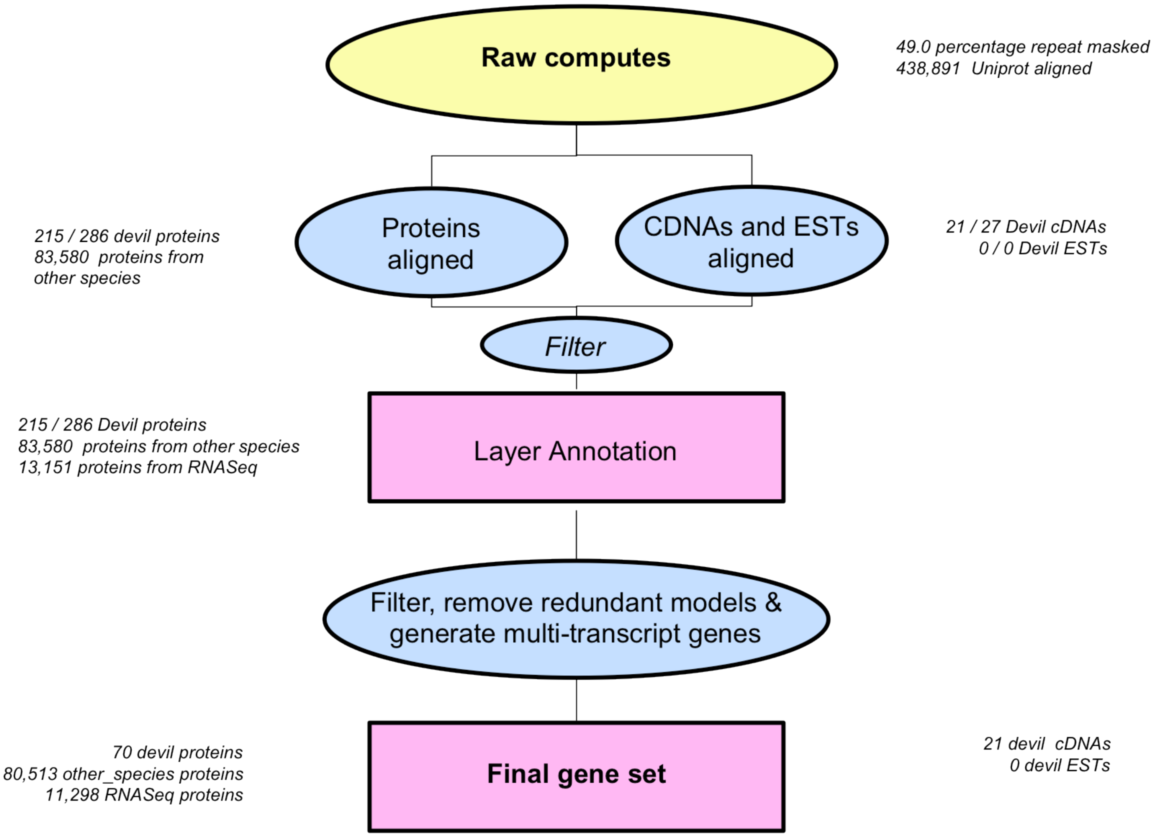
Ensembl gene annotation project

Sarcophilus Harrisii (Tasmanian Devil)

Raw Computes Stage: Searching for sequence patterns, aligning proteins and cDNAs to the genome.

The annotation process of the high-coverage Tasmanian Devil assembly began with the “raw compute” stage [Figure 1] whereby the genomic sequence was screened for sequence patterns including repeats using RepeatMasker [1] version 3.2.8), Dust [2] and TRF [3]. RepeatMasker and Dust combined masked 49.0% of the devil genome.



49.0 percentage repeat masked
438,891 Uniprot aligned

215 / 286 devil proteins
83,580 proteins from other species

21 / 27 Devil cDNAs
0 / 0 Devil ESTs

215 / 286 Devil proteins
83,580 proteins from other species
13,151 proteins from RNASeq

70 devil proteins
80,513 other_species proteins
11,298 RNASeq proteins

21 devil cDNAs
0 devil ESTs

Figure 1: Summary of devil gene annotation project.

Transcription start sites were predicted using Eponine-scan [4], and FirstEF [5] CpG islands and tRNAs [6] were also predicted.

Genscan [7] was run across RepeatMasked sequence and the results were used as input for UniProt [8], UniGene [9], and Vertebrate RNA [10] alignments by WU-BLAST [11] (Passing only Genscan results to BLAST is an effective way of reducing the search space and therefore the computational resources required). This resulted in 438,891 UniProt , 316,010 UniGene and 309,061 Vertebrate RNA sequences aligning to the genome.

Targeted Stage: Generating coding models from devil evidence

Devil protein sequences were downloaded from public databases (UniProt SwissProt/TrEMBL [8] and Genbank) and filtered to remove sequences based on predictions. The devil sequences were mapped to the genome using Pmatch as indicated in Figure 2.

Models of the coding sequence (CDS) were produced from the proteins using Genewise [13]. 2 sets of models were produced, one with only consensus splice sites and one where non-consensus splices were allowed; where a single protein sequence had generated two different coding models at the same locus, the BestTargetted module was used to select the coding model that most closely matched the source protein to take through to the next stage of the gene annotation process. The generation of transcript models using devil-specific data is referred to as the "Targeted stage". This stage resulted in 215 of 286 devil proteins used to build 215 coding models.

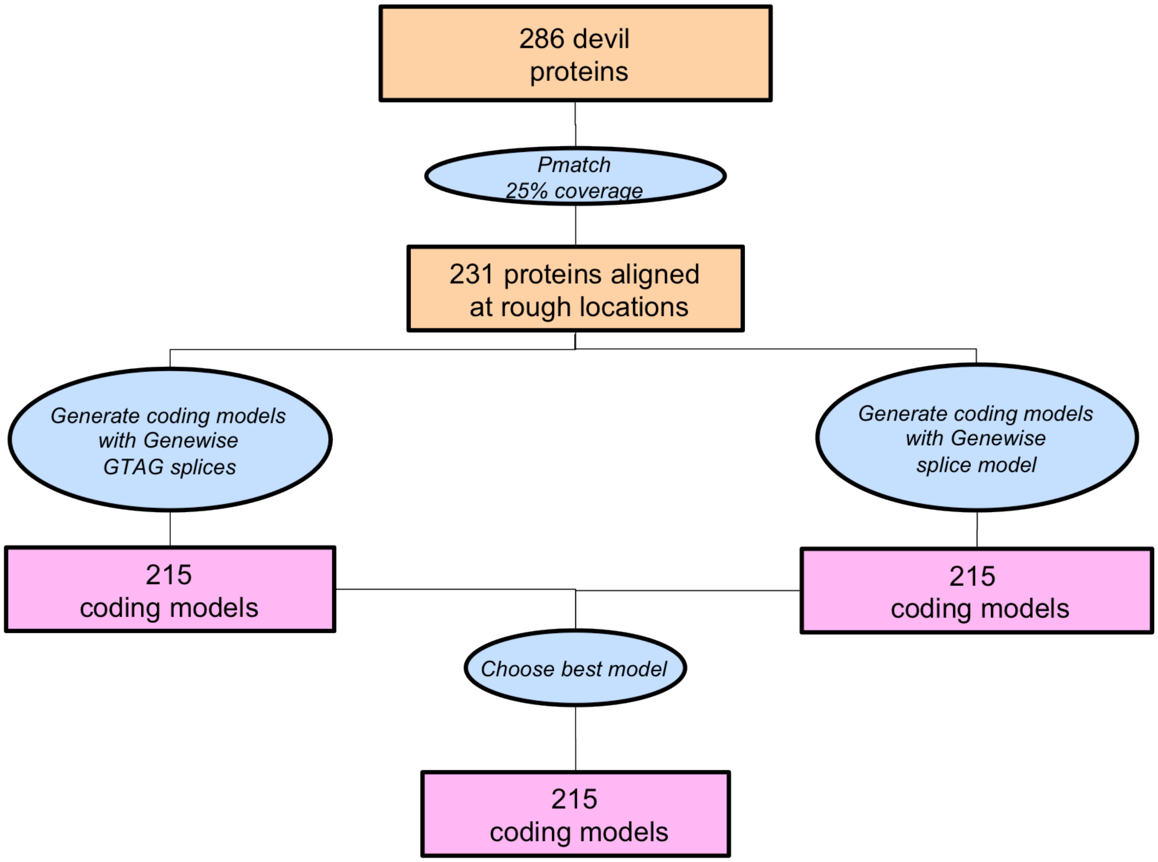

286 devil proteins
Pmatch 25% coverage
231 proteins aligned at rough locations
Generate coding models with Genewise GTAG splices
Generate coding models with Genewise splice model
215 coding models
215 coding models
Choose best model
215 coding models

Figure 2: Targeted stage using devil specific proteins

cDNA and EST Alignment

Devil cDNAs were downloaded from Genbank, clipped to remove polyA tails, and aligned to the genome using Exonerate. Of these, 21 of 27 devil cDNAs aligned with a cut-off of 90% coverage and 97% identity. No ESTs were found.

Similarity Stage: Generating additional coding models using proteins from related species

Due to the small number of devil specific protein and cDNA evidence the majority of the gene models were based on proteins from other species. UniProt alignments from the Raw Compute step were filtered to favor proteins classed by UniProt's Protein Existence (PE) classification level 1 and 2. Proteins from other PE levels were used where no other evidence was available; similarly, mammalian proteins were favored over non-mamalian. WU-BLAST was rerun for these sequences and the results were passed to Genewise to build coding models. The generation of transcript models using data from related species is referred to as the "Similarity stage". This stage resulted in 109,194 coding models.

Filtering Coding Models

Coding models from the Similarity stage were filtered using modules such as TranscriptConsensus, RNA-Seq spliced alignments supporting introns were used to help filter the set. 61,937 models were rejected as a result of filtering. The Apollo software [15] was used to visualise the results of filtering. [Figure 3]

Addition of RNA-Seq models

The largest set of devil specific evidence was from Illumina paired end RNASeq, this was used where appropriate to help inform our gene annotation. A set of 1.6 billion reads was aligned to the genome using BWA resulting in 1.25 billion reads aligning and properly pairing. The Ensembl RNA-Seq pipeline was used to process the BWA alignments and create a further 86 million split read alignments using Exonerate. The split reads and the processed BWA alignments were combined to produce 41,011 transcript models in total; one transcript per loci. The predicted open reading frames were compared to Uniprot Protein Existence (PE) classification level 1 and 2 proteins using WU-BLAST, models with no BLAST alignment or poorly scoring BLAST alignments were discarded. The resulting models were added into the gene set where they produced a novel model or splice variant, in total 5,663 models were added.

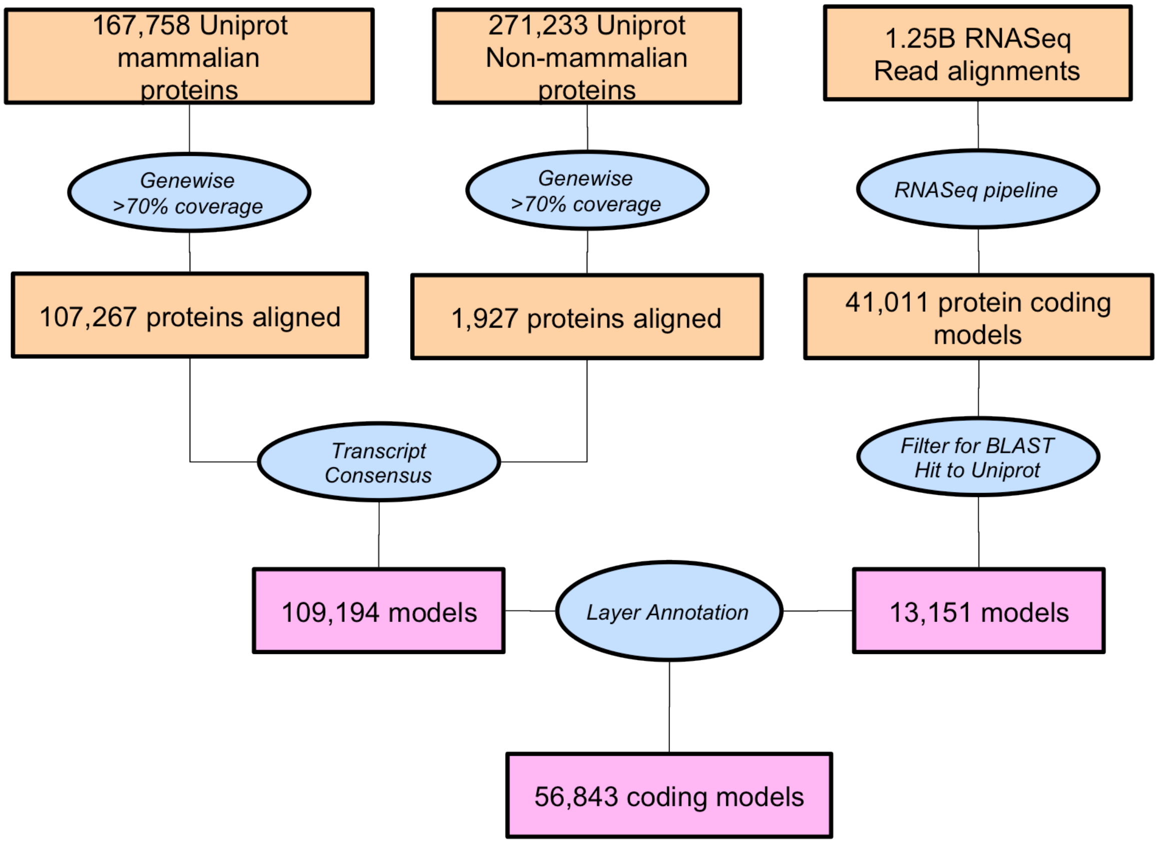


Figure 3: Alignment and filtering of other species proteins and addition of RNASeq models

Generating multi-transcript genes

The above steps generated a large set of potential transcript models, many of which overlapped one another. Redundant transcript models were removed and the remaining unique set of transcript models were clustered into multi-transcript genes where each transcript in a gene has at least one coding exon that overlaps a coding exon from another transcript within the same gene. The final gene set of 18,775 genes included 2 genes built only using devil proteins, a further 13,095 genes built only using proteins from other species, and 3,019 genes built only from RNA-Seq evidence. 2,644 genes had a mixture of RNA-Seq and other species proteins and 15 genes were a mixture of devil specific proteins and proteins from other species.

Pseudogenes, Protein annotation, non-coding genes, Cross referencing, Stable Identifiers

The gene set was screened for potential pseudogenes. Before public release the transcripts and translations were given external references cross references to external databases), while translations were searched for domains/signatures of interest and labeled where appropriate. Stable Identifiers were assigned to each gene, transcript, exon and translation. (When annotating a species for the first time, these identifiers are auto-generated. In all subsequent annotations the stable identifiers are propagated based on comparison of the new gene set to the previous gene set.)

Small structured non-coding genes were added using annotations taken from RFAM [16] and miRBase [17].

The final gene set consists of 18,775 protein coding genes containing 22,391 transcripts, 178 pseudogenes and 1,446 ncRNAs. 5,663 transcripts were made from RNASeq, 16,711 transcripts came from proteins from other species with 8,692 of them having UTR added from RNASeq. 17 transcripts were devil specific.

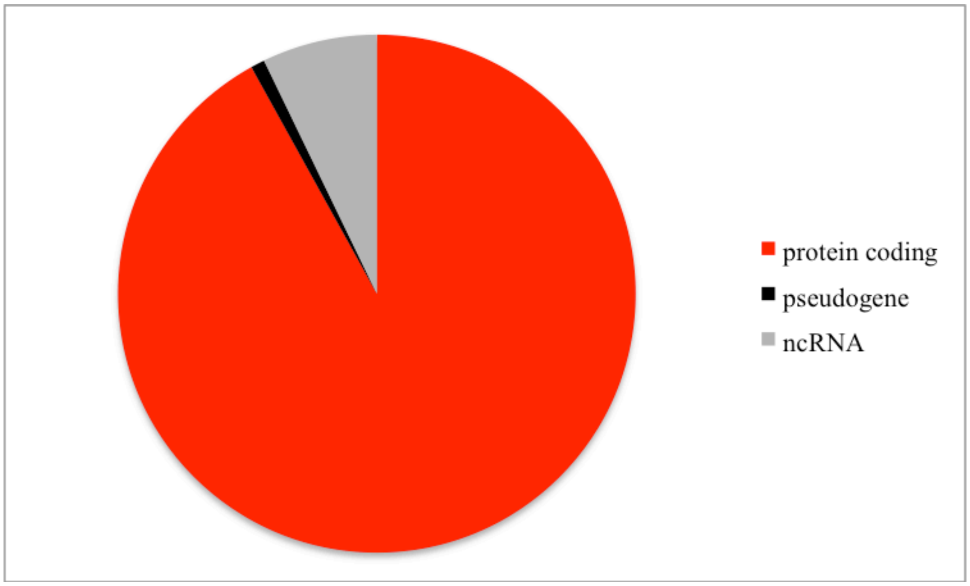


Figure 4: Composition of devil gene set.

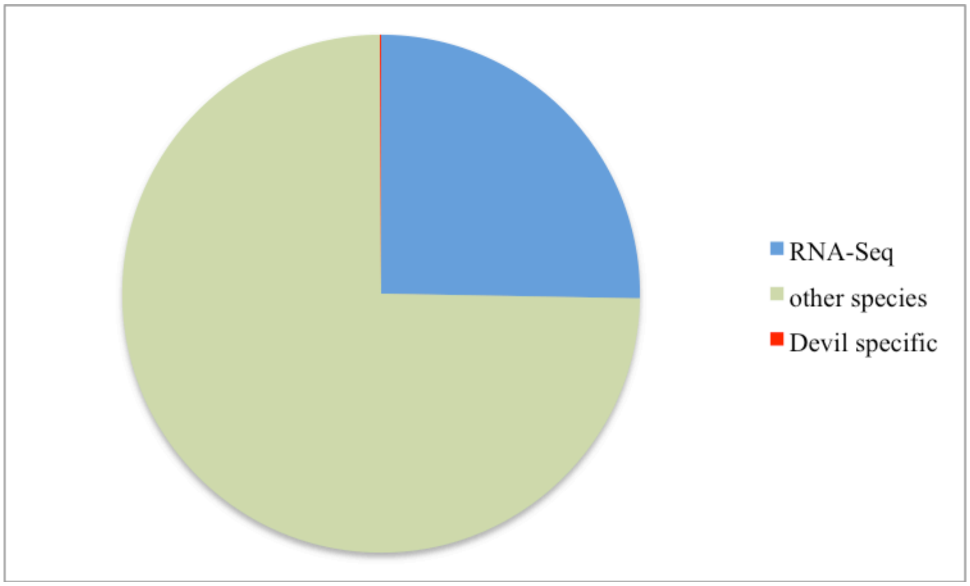


Figure 5: Composition of devil transcripts

Further information

The Ensembl gene set is generated automatically, meaning that gene models are annotated using the Ensembl gene annotation pipeline. The main focus of this pipeline is to generate a conservative set of protein-coding gene models, although non-coding genes and pseudogenes may also annotated.

Every gene model produced by the Ensembl gene annotation pipeline is supported by biological sequence evidence (see the "Supporting evidence" link on the left-hand menu of a Gene page or Transcript page); ab initio models are not included in our gene set. Ab initio predictions and the full set of cDNA and EST alignments to the genome are available on our website.

The quality of a gene set is dependent on the quality of the genome assembly. Genome assembly can be assessed in a number of ways, including:

1. Coverage estimate

- A higher coverage usually indicates a more complete assembly.
- Using Sanger sequencing only, a coverage of at least 2x is preferred.

2. N50 of contigs and scaffolds
    - A longer N50 usually indicates a more complete genome assembly.
    - Bearing in mind that an average human gene may be 10-15 kb in length, contigs shorter than this length will be unlikely to hold full-length gene models.
3. Number of contigs and scaffolds
    - A lower number toplevel sequences usually indicates a more complete genome assembly.
4. Alignment of cDNAs and ESTs to the genome
    - A higher number of alignments, using stringent thresholds, usually indicate a more complete genome assembly.

More information on the Ensembl automatic gene annotation process can be found at:

• Curwen V, Eyras E, Andrews TD, Clarke L, Mongin E, Searle SM, Clamp M. The Ensembl automatic gene annotation system. Genome Res. 2004, 14(5):942-50. [PMID: 15123590]

• Potter SC, Clarke L, Curwen V, Keenan S, Mongin E, Searle SM, Stabenau A, Storey R, Clamp M. The Ensembl analysis pipeline. Genome Res. 2004, 14(5):934-41. [PMID: 15123589]

• http://www.ensembl.org/info/docs/genebuild/genome_annotation.html

* http://cvs.sanger.ac.uk/cgi-bin/viewvc.cgi/ensembldoc/pipeline_docs/the_genebuild_process.txt?root=ensembl&view=co

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